

MYCOFLORA ASSOCIATED WITH GREEN LEAVES AND LEAF LITTER OF *QUERCUS GERMANA*, *QUERCUS SARTORII* AND *LIQUIDAMBAR STYRACIFLUA* IN A MEXICAN CLOUD FOREST

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ABSTRACT - The mycoflora of green leaves and leaf litter of *Quercus germana*, *Q. sartorii* and *Liquidambar styraciflua* was studied by damp chambers, isolations from surface-sterilized and washed leaf fragments. The three types of leaves shared a high fungal species percentage. Dominant species on green leaves were different from those on leaf litter. On green leaves, *Pestalotiopsis maculans* was the most frequently recorded species from the three leaf specimens. In decaying litter most of the fungi were sporadic and had low frequency of occurrence. Only the fungi *Beltrania rhombica*, *Codinaea assamica*, *Chalara alabamensis*, *Cryptophiale kakombensis*, *Cylindrocladium scoparium*, *Ellisiopsis gallsiae* and *Subulispora procurvata* were detected throughout the decomposition process. *Beltrania rhombica* was the dominant fungus on *Q. germana* and *Q. sartorii* leaf litter and *C. scoparium* on *L. styraciflua* leaf litter. The general pattern of succession scheme was similar to that reported from leaves of other species of deciduous trees. The leaf litter fungi were characteristic of tropical ecosystems.

RÉSUMÉ - La mycoflore des feuilles vertes et de la litière de *Quercus germana*, *Quercus sartorii* et *Liquidambar styraciflua* a été étudiée à l'aide de chambres humides, et isolée des feuilles après stérilisation de la surface et lavages sériés de fragments. Les 3 sortes de feuilles partagent un grand pourcentage d'espèces fongiques. Les espèces dominantes sur les feuilles vertes sont différentes de celles de la litière. Sur les feuilles vertes, l'espèce la plus abondante est *Pestalotiopsis maculans*. Dans la litière en décomposition, la plupart des champignons sont sporadiques et peu abondants. Seules les espèces *Beltrania rhombica*, *Chalara alabamensis*, *Codinaea assamica*, *Cryptophiale kakombensis*, *Cylindrocladium scoparium*, *Ellisiopsis gallsiae* et *Subulispora procurvata* sont observées tout au long du processus de décomposition. *Beltrania rhombica* est le champignon le plus abondant sur la litière de *Q. germana* et *Q. sartorii* et *C. scoparium* sur celle de *L. styraciflua*. Le schéma général de succession des espèces est semblable à celui des feuilles d'arbres décidues. Les champignons de ces litières sont caractéristiques d'écosystèmes tropicaux.

KEY WORDS : Mycoflora, leaves, litter, *Quercus germana*, *Q. sartorii*, *Liquidambar styraciflua*, Mexico.

INTRODUCTION

Due to their saprophytic ability, fungal communities play an important role in the decomposition of plant and animal residues and therefore in the release of nutrients (Harley, 1971). In forests, leaves are the major components of litter (Jensen, 1974).

Long before leaves fall, the process of decay begins. Green leaves surfaces form suitable substrates for different kinds of saprophytic and parasitic fungi. When leaf abscission takes place and during leaf decomposition above the soil surface, one group of fungi may be replaced by another group. The sequential appearance of fungal species on plant residues is considered as a fungal succession. This phenomenon has been widely studied on many different substrates.

A detailed review of the studies dealing with fungal successions on leaf litter was published by Hudson (1968). Most work has been focused on temperate tree leaves like oak, birch, hazel, ash (Hering, 1965), beech (Hogg & Hudson, 1966) and poplar (Visser & Parkinson, 1975).

Little is known about the mycoflora and successional patterns on tropical plant residues. Hudson (1962) studied fungal succession on ageing leaves of sugar cane and Meredith (1962) isolated fungi on banana leaves. For wild tropical species, Kiffer et al. (1981) described the fungal succession throughout the decomposition of leaf litter of *Euperia falcata* in French Guyana and Rambelli et al. (1983) published an extensive study on the leaf litter fungi from a tropical rain forest in the South Western Ivory Coast.

In Mexico no studies on the green leaves and leaf litter mycoflora have so far been carried out. The cloud forest is interesting to be studied. The mixture of tropical and temperate tree species and the high accumulation of leaf litter make this kind of forest suitable for studies dealing with leaf litter fungi.

The aims of this work were: (a) to determine the mycoflora on green and decaying leaves of three dominant tree species (*Liquidambar styraciflua* L., *Quercus sartorii* Liemb., and *Quercus germana* Cham. et Schlecht) in a Mexican cloud forest, (b) to assess the frequency of the dominant fungal species in the leaf litter during decomposition, (c) to describe the fungal succession occurring during the decay of the leaf litter.

STUDY AREA

The study was performed in a cloud forest located within the "El Cielo" Biosphere Reserve in Northeastern Mexico. A detailed description of the site is given by Sosa (1987). The sampling area is at an altitude of 1200 m. The annual mean temperature is 13.8°C being highest in May (34.4°C) and lowest in January and February (-2.2°C), with a total annual rain-fall of 2522 mm. (Fig. 1) (Puig & Bracho, 1987).

Forest structure has been studied by Puig et al. (1987). *Liquidambar styraciflua* L., *Quercus sartorii* Liemb., *Quercus germana* Cham. et Schlecht and *Clethra pringlei* S. Watson are the dominant trees. Understorey includes ferns, mosses, hepatics and selaginellas.

Bracho & Puig (1987) studied litter production in several areas of the cloud forest. Litterfall biomass was estimated at 7.3 ton/ha/year, 5.6 ton/ha/year (76.7%) being composed of leaves. The species with the greatest contributions were the deciduous trees *Q. sartorii*, *Q. germana* and *L. styraciflua*. These authors reported two peaks of leaf fall, in January and November, but leaf fall may occur throughout the year due to frequent winds in this area. The litter layer on soil is 5 to 10 cm depth.

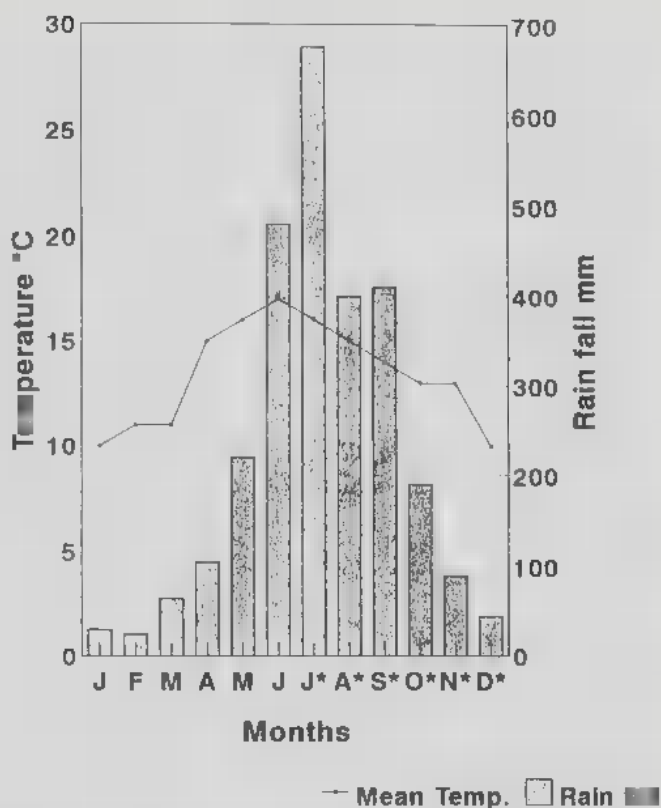


Fig. 1 - Mean monthly temperature and rainfall in study area. * Sample dates.

Fig. 1 - Moyenne mensuelle des températures et des précipitations de l'aire d'étude. * Date d'échantillonnage.

MATERIAL AND METHODS

Collection of leaf litter.

In July freshly fallen leaves of *Q. germana*, *Q. sartorii* and *L. styraciflua* were collected from the ground. Leaves were placed in litter bags (20 x 25 cm, 3 mm mesh) (Bocock & Gilbert, 1957). Each bag contained 20 leaves. For each type of leaf 30 litter bags were made up. The bags were deposited within three plots on top of the natural litter cover. Sampling dates were 30 Aug., 7 Oct., 28 Oct., 29 Nov., and 22 Dec. At each collection-date, two randomly chosen bags of each species were collected from each plot (six replicates). Samples were placed separately in sterile polyethylene bags and stored at 5°C before processing.

Leaf disks were punched from each leaf with a sterile cork borer of 5 mm diam. One half of each batch of disks was used for isolation in culture medium and the remainder was used for damp-chamber incubations.

Isolation in culture medium.

The isolations were carried out by means of serially washing of previously surface-sterilized leaf disks. This method was used to isolate fungi growing in the leaf tissues. Leaf disks were surface sterilized in sodium hypochlorite for 1 min and then washed 10 times with sterile water for 1 min. Between washings, the vials were completely drained. For each sample, 50 leaf disks were transferred to 10 Petri dishes containing potato-dextrose-agar (PDA) with 0.03 gr streptomycin (Ulloa & Harlin, 1978).

Plates were incubated at 25°C and examined after 8 days. Representative isolations of fungal colonies were made for further identification.

Damp chamber incubation.

For each sample, 50 disks were distributed in 5 damp chambers. The chambers ■ sterile Petri dishes with filter papers, which were kept moistened by additions of sterilized water every 3-5 days. Incubation was at 25°C for 30 days. Microscopic examination was made by using pieces of double-adhesive tape (Langvard, 1980). Tapes were stained with lactophenol-cotton blue.

At the initiation of the study (in July), living green leaves were collected from the three tree species studied. Mycological observations were made by the same methods described for the leaf litter. For each tree species 500 leaves were collected, from which 250 disks were incubated in damp chambers and 200 were used for fungal isolations.

For each fungal species growing in damp-chambers and on PDA medium, the percentage frequency of occurrence defined as the number of leaf-disks on which fungus occurred / total number of disks plated x 100 was recorded.

Means of the percentage of occurrence for each fungus were calculated from the six replicates. The Mann-Whitney and Kruskal Wallis tests were applied to test for the significance of variation among sampling dates (Zar, 1974). The Sorensen Index was used to compare the similarity of the mycoflora composition of the three leaf species (Brower & Zar, 1977).

Isolates were identified by means of standard mycological methods (Barron 1968; Carmichael et al., 1980; Ellis, 1971, 1976; Glawe & Crane, 1987; Morgan-Jones & Ingram, 1976; Nag Raj & Kendrick, 1975; Nag Raj 1985; Nawawi & Kuthubutheen, 1987; Prozynski, 1963; Rifai, 1969).

RESULTS

Table 1 shows the fungal species observed in damp chambers and isolated by serial washing. More species were detected by damp chambers than by cultural isolations. The percentage frequency of occurrence of the most representative fungal species for the three kinds of leaves studied are shown in Tables 3, 4 and 5.

Green leaves mycoflora.

The three types of leaves had many common fungal species (Table 2). Most of the species observed on green leaves disappeared in the leaf litter during the early stages of decomposition. Only the fungi *Beltrania rhombica* and *Pestalotiopsis maculans* were detected on green leaves ■ well as on leaves in early and late stages of decay.

SPECIES	GREEN LEAVES			LEAF LITTER			METHOD	
	Q.g.	Q.s.	L.s.	Q.g.	Q.s.	L.s.	A	B
<i>Alternaria alternata</i> (Fr.) Keissler	+	+	+	+	+	+	+	+
<i>A. tenuissima</i> (Kunze ex Pers.) Wiltshire	+	+	+	+	+	+	+	+
<i>Aspergillus fumigatus</i> Fres.	+	+	+	+	+	+	+	+
<i>Aspergillus niger</i> van Tieghem	-	-	-	+	+	+	+	+
<i>Beltrania guerna</i> Warknes	+	+	+	+	+	-	+	-
<i>Beltrania rhombica</i> O. Penzig	+	+	+	+	+	+	+	+
<i>Cercospora</i> sp	+	+	+	-	-	+	+	-
<i>Curularia lunata</i> (Walker) Boedijn	+	+	+	-	-	+	+	-
<i>Cylindrocidium scoparium</i> Morgan	-	-	-	+	+	+	+	+
<i>Cladosporium cladosporioides</i> (Fresen.) de Vries	+	+	+	+	+	+	+	+
<i>C. herbarum</i> (Pers.) Link ■ S.F. Gray	+	+	+	+	+	+	+	+
<i>Codinaea asseptica</i> (Ag.) Hughes et Kendrick	+	+	-	+	+	+	+	+
<i>Codinaea simplex</i> Hughes et Kendrick	-	-	-	+	+	-	+	-
<i>Cryptophiala kakabensis</i> Pirozynski	-	-	-	+	+	+	+	-
<i>Chaetomium globosum</i> Kunze:Fr.	-	-	-	+	+	+	-	+
<i>Chalara alabamensis</i> Morgan-Jones et Ingram	-	-	-	+	+	+	+	-
<i>Chalara urceolata</i> Nag Raj et Kendrick	-	-	-	+	+	-	+	-
<i>Dactylella ellisospora</i> [L.S.]	-	-	-	-	-	+	+	-
<i>Dendrosporium lobatum</i> (Plakidias et Edgerton)	-	-	-	-	-	+	+	-
<i>Dictyosporium heptasporum</i> (Garov.) Damon	-	-	-	+	-	-	+	-
<i>Diplocladiella scalaroides</i> Arnaud	-	-	-	+	-	+	+	-
<i>Drechslera hawaiiensis</i> Subram et Sain	-	-	-	+	+	+	+	+
<i>Ellisiopsis gallsiae</i> Batista et Nascimento	+	+	+	+	+	+	+	+
<i>Epicoccum purpurascens</i> Ehrenb	+	+	+	+	+	+	+	+
<i>Fusarium</i> ■	-	-	-	+	+	+	+	+
<i>Hemicola</i> sp	-	-	-	+	-	-	+	-
<i>Idriella lunata</i> Nelson et Wilbalm	+	-	-	+	-	-	+	-
<i>Neurospora theobromae</i> Hughes	-	-	-	-	+	-	+	-
<i>Monochaetia</i> sp	-	-	+	-	-	-	+	-
<i>Nigrospora sphaerica</i> (Sacc.) Masson	-	-	-	-	+	-	+	-
<i>Nucor hiemalis</i> Vehner	-	-	-	+	+	+	+	+
<i>Olpitrichum macrosporium</i> Atkinson	+	-	-	-	-	-	+	-
<i>Penicillium</i> spp	-	-	-	+	+	+	+	+
<i>Pestalotiopsis maculans</i> (Cda.) Hughes	+	+	+	+	+	+	+	+
<i>Phialocephala</i> sp	-	-	-	+	+	-	+	-
<i>Phoma</i> ■	+	+	-	+	+	-	+	+
<i>Pithomyces chartarum</i> (Berk. et Curt.) Ellis	+	-	-	+	+	-	+	-
<i>Rhinocladia</i> sp	-	+	+	-	-	-	+	-
<i>Stachybotrys atra</i> Corda	-	-	-	-	-	+	+	+
<i>Subulispora procurvata</i> Tubaki	-	-	-	+	+	+	+	-
<i>Tetraploa aristata</i> Berk ■ Br.	+	+	-	-	-	-	+	-
<i>Trichoderma viride</i> Pers.	-	-	-	+	+	+	+	+
<i>Triposperium myrti</i> (Lind.) Hughes	+	+	+	-	-	-	+	-
<i>Triscelosporium verrucosum</i> Nawawi & Ruth	-	-	-	+	-	-	+	-
<i>Tubakia dryina</i> (Sacc.) Sutton	+	+	-	+	+	-	+	-
<i>Verticillium</i> spp	-	-	-	+	-	+	-	-

A = Moist chamber (chambre humide) B = Washed disks (disques lavés)
+ present; - absent

Table 1 - Fungal species present on green leaves and leaf litter of *Quercus germana* (Q. g.), *Quercus sartorii* (Q. s.) and *Liquidambar styraciflua* (L. s.).

Tableau 1 - Champignons présents sur les feuilles vertes et la litière de *Quercus germana* (Q. g.), *Quercus sartorii* (Q. s.) et *Liquidambar styraciflua* (L. s.).

The most frequently recorded species on green leaves were *P. maculans* on *Q. germana* and *L. styraciflua*, and *Tubakia dryina* on *Q. sartorii* in damp chambers. In cultural isolations, *P. maculans* was the fungus with the highest percentage of occur-

rence for the three leaf species. The fungi restricted to green leaves were *Olpitrichum macrosporum* on *Q. germana*, *Rhinochadiella* sp. on *Q. sartorii*, and *L. styraciflua* and *Tripaspermium myrti* on leaves of the three species.

Leaf litter mycoflora.

As with the green leaf mycoflora, a high percentage of shared species was observed on leaves of the three tree species (Table 2). Throughout the decomposition period, only a few species were present at all samplings. These species exhibited sharp changes in their frequency of occurrence from one date to the other.

Q. germana leaves suffered the least changes during decay, although leaves became progressively darker in color. After 104 days, many leaves showed few changes in shape, at the end of the study 60% of the samples were fragmented.

The fungi most frequently recorded on *Q. germana* fresh fallen leaves were *B. rhombica* and *S. procurvata* (Table 3). No significant difference was found in their frequency of occurrence ($P=0.388$ Mann-Whitney U). On subsequent samplings, the fungus *B. rhombica* became the dominant species. Significant differences in its frequency of occurrence were found as decomposition advanced ($P=0.012$ Kruskal-Wallis).

Q. sartorii leaves turned dark in very early decomposition stages and by the end of the study all leaves were fragmented. In Table 4 data of the mycoflora on *Q. sartorii* are presented. Like in *Q. germana*, on fresh fallen leaves and in early decomposition stages, *B. rhombica* was the most frequently recorded species.

In later samplings besides *B. rhombica*, the fungi *E. galesiae* and *Ch. alabamensis* were the most prominent species. After 74 days there were no significant differences between *B. rhombica* and *E. galesiae* ($P=0.500$ Mann-Whitney U). At 102 days even though *Ch. alabamensis* showed higher percentages than *B. rhombica*, no significant difference was found in their frequency of occurrence ($P=0.196$ Mann-Whitney U). During late stages of decay (134 and 157 days) *B. rhombica* was the dominant species again.

L. styraciflua leaves were the most susceptible to decay. The leaves darkened rapidly and most of the leaf material had disappeared by the end of the study. The dominant fungus on *L. styraciflua* was *C. scoparium* (Table 5). In intermediate decay stages, some species of *Fusarium* were observed. During late stages of decay, an increase in *Trichoderma viride* was observed on the isolation plates.

Tree leaf species	Green leaves	Leaf litter
<i>Q. germana</i> vs <i>Q. sartorii</i>	89%	86%
<i>Q. germana</i> vs <i>L. styraciflua</i>	75%	72%
<i>L. styraciflua</i> vs <i>Q. sartorii</i>	65%	73%

Table 2 - Similarity among the mycoflora of *Quercus germana*, *Quercus sartorii* and *Liquidambar styraciflua* green leaves and leaf litter, according to the Sorensen Index.

Tableau 2 - Similarité de la mycoflore entre feuilles vertes et de litière de *Quercus germana*, *Quercus sartorii* et *Liquidambar styraciflua* selon l'indice de Sorensen.

SPECIES	Green leaves		TIME (Days)											
			0*		45		74		102		134		157	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<i>Alternaria</i> spp	20	14	14	6	6	6	-	-	-	-	-	-	-	-
<i>Beitrania rhombica</i>	8	4	56	46	27	61	74	76	87	55	62	51	68	60
<i>Cladosporium</i> spp	27	13	23	6	6	4	-	-	-	-	-	-	-	-
<i>Codinaea assamica</i>	17	-	-	-	21	-	39	-	10	-	20	-	18	1
<i>Cylindr. scoparium</i>	-	-	10	5	18	9	6	11	6	8	8	12	-	12
<i>Crypt. kakombensis</i>	-	-	-	-	18	-	16	-	16	-	16	-	9	1
<i>Chala. alabamensis</i>	-	-	25	-	18	-	37	-	56	-	10	-	-	-
<i>Ellisia. galleisae</i>	9	-	28	-	8	-	26	10	10	5	38	10	-	-
<i>Epic. purpurascens</i>	12	20	7	3	-	-	-	-	-	-	-	-	-	-
<i>Fusarium</i> spp	-	-	-	-	-	-	-	9	-	8	9	8	18	12
<i>Mucor hiemalis</i>	-	-	-	-	-	-	-	-	7	-	10	-	5	-
<i>Penicillium</i> spp	-	-	-	-	-	-	-	-	10	8	7	19	16	-
<i>Pestalot. maculans</i>	44	28	25	17	-	14	-	7	-	7	-	5	-	-
<i>Sub. procurvata</i>	-	-	50	-	19	-	10	-	2	-	3	-	14	-
<i>Trichoderma viride</i>	-	-	-	-	15	27	11	45	7	36	9	38	27	38
<i>Tubakia dryina</i>	28	1	15	-	18	-	-	-	-	-	-	-	-	-
<i>Verticillium</i> sp	-	-	-	-	-	-	-	-	2	-	-	-	28	16

- Fresh fallen leaves
- Feuilles récemment tombées

Table 3 - Percentage frequency of observed fungi in moist chambers (A) and isolated from disinfected-washed fragments (B) from green leaves and leaf litter of *Quercus germana*. Values are the average of six replicates. Only those species occurring with a frequency higher than 5% have been listed.

Tableau 3 - Pourcentage de fréquence des champignons observés dans les chambres humides (A) et isolés des fragments désinfectés-lavés (B) des feuilles vertes et de la litière de *Quercus germana*. Les valeurs sont la moyenne de 6 répétitions. Seules les espèces ayant une fréquence supérieure à 5% sont présentées.

SPECIES	Green leaves		TIME (Days)											
			0*		45		74		102		134		157	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<i>Alternaria</i> spp	14	11	17	3	9	6	6	-	-	-	-	-	-	-
<i>Beitrania rhombica</i>	9	-	61	42	80	42	56	52	48	27	64	33	87	27
<i>Cladosporium</i> spp	13	5	20	6	10	4	10	-	-	-	-	-	-	-
<i>Codinaea assamica</i>	5	-	-	-	16	-	16	-	24	-	27	-	26	-
<i>Crypt. kakombensis</i>	-	-	-	-	8	-	8	-	6	-	16	-	10	-
<i>Cylindr. scoparium</i>	-	-	-	14	3	15	5	5	12	7	10	5	10	7
<i>Chala. alabamensis</i>	-	-	-	-	12	-	30	-	68	-	47	-	67	-
<i>Ellisia. galleisae</i>	10	-	13	-	28	-	59	-	45	-	20	-	-	-
<i>Fusarium</i> spp	3	3	4	3	16	10	-	-	-	-	11	3	8	7
<i>Mucor hiemalis</i>	-	-	-	-	-	-	-	-	-	-	8	-	9	-
<i>Pestalot. maculans</i>	16	28	-	3	4	5	11	5	10	3	4	1	11	7
<i>Penicillium</i> spp	-	-	-	-	-	-	-	3	-	7	-	3	-	18
<i>Sub. procurvata</i>	-	-	6	-	10	-	10	-	13	-	17	-	17	-
<i>Trichoderma viride</i>	-	-	-	6	-	13	13	20	26	30	17	25	10	57
<i>Tubakia dryina</i>	49	-	12	-	13	-	-	-	-	-	-	-	-	-

- Fresh fallen leaves
- * Feuilles récemment tombées

Table 4 - Percentage frequency of observed fungi in moist chambers (A) and isolated from disinfected-washed fragments (B) from green leaves and leaf litter of *Quercus sartorii*. Values are the average of six replicates. Only those species occurring with a frequency higher than 5% have been listed.

Tableau 4 - Pourcentage de fréquence des champignons observés dans les chambres humides (A) et isolés des fragments désinfectés-lavés (B) des feuilles vertes et de la litière de *Quercus sartorii*. Les valeurs sont la moyenne de 6 répétitions. Seules les espèces ayant une fréquence supérieure à 5% sont présentées.

SPECIES	Green leaves		TIME (Days)											
			0*		45		74		102		134		157	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<i>Alternaria</i> spp	21	7	15	5	10	3	5	3	-	-	-	-	-	-
<i>Asp. fumigatus</i>	-	-	-	-	-	-	-	-	10	5	-	7	-	10
<i>Beltrania rhombica</i>	5	-	40	20	-	-	-	-	-	-	-	-	-	-
<i>Cladosporium</i> spp	10	15	20	10	7	15	5	5	-	-	-	-	-	-
<i>Codinaea assamica</i>	-	-	-	-	6	-	2	-	29	-	33	-	-	-
<i>Cylindro. scoparium</i>	-	-	-	-	80	28	56	97	47	91	70	66	83	76
<i>Chaia. siabamensis</i>	-	-	18	-	13	-	17	-	28	-	-	-	-	-
<i>Ellisio. gallestiae</i>	-	-	34	-	12	-	-	-	-	-	-	-	-	-
<i>Epic. purpurascens</i>	17	-	4	4	-	-	-	-	-	-	-	-	-	-
<i>Fusarium</i> spp	-	-	-	-	26	19	18	20	15	10	9	12	-	-
<i>Mucor hiemalis</i>	-	-	-	-	-	-	-	-	-	-	17	-	11	-
<i>Penicillium</i> spp	-	-	-	-	3	-	5	-	8	-	10	25	7	17
<i>Pestalot. maculans</i>	80	47	46	25	-	-	-	-	-	-	-	-	-	-
<i>Sub. procurvata</i>	-	-	33	-	12	-	16	-	10	-	-	-	-	-
<i>Trichoderma viride</i>	-	-	-	-	17	-	14	-	32	62	43	56	43	56
<i>Verticillium</i> sp	-	-	-	-	-	-	-	-	16	9	7	5	21	13

• Fresh fallen leaves

■ Feuilles récemment tombées

Table 5 - Percentage frequency of observed fungi in moist chambers (A) and isolated from disinfected-washed fragments (B) from green leaves and leaf litter of *Liquidambar styraciflua*. Values are the average of six replicates. Only those species occurring with a frequency higher than 5% have been listed.

Tableau 5 - Pourcentage de fréquence des champignons observés dans les chambres humides (A) et isolés des fragments désinfectés-lavés (B) des feuilles vertes et de la litière de *Liquidambar styraciflua*. Les valeurs sont la moyenne de 6 répétitions. Seules les espèces ayant une fréquence supérieure à 5% sont présentées.

DISCUSSION

The importance of using complementary techniques in mycological studies has been pointed out by Dickinson (1971). In spite of the limitations of the two methods employed in this study, they provided enough information to compare the mycoflora between green leaves and leaf litter and to detect changes in the fungal species involved in the decomposition of the three leaf species.

Very few species observed in damp chambers were isolated by the washing and plating technique. This could be due to the difficulty of these fungi to compete with the fast-growing species when plated on synthetic media.

The fungi observed on sterilized surface washed disks may be considered as internal tissue colonizers involved in the depletion of the substrate. The frequent occurrence of *Trichoderma viride* on plates could be related to the low number of species observed in the cultural isolations. This fungus covered the whole surface of some plates, preventing the growth of other species.

Examination of green leaves revealed the species capable of surviving on leaf litter. The fungi *Alternaria* spp., *Cladosporium* spp. and *E. purpurascens* were found on green leaves and litter in the early stages of decay. These species are common leaf-inhabitants and have been reported in the phylloplane of a wide range of plants. Because of their frequency on senescent leaves and relatively undecayed plant debris, these species are considered as common primary saprophytes (Hudson, 1968).

Pestalotiopsis maculans was one of the few fungi found on green leaves and during all stages of litter decay. This fungus presented a high frequency occurrence on both green leaves and fresh fallen leaves. *Pestalotiopsis* spp. have been reported as early colonizers on the leaf litter of *Nothofagus truncata* (Ruscoe, 1971), on *Quercus phillyraeoides* and *Castanopsis cuspidata* (Tubaki & Yokoyama, 1971). *Pestalotiopsis* spp. are known plant parasites on numerous species of higher plants (Guba, 1961). Its presence, even in low percentages, on well decayed leaves demonstrates its ability to survive on dead material.

The litter mycoflora on leaves of the three tree species was characterized by many fungi that appeared sporadically and with low frequency. Only a few species were constant throughout the decomposition process. A similar observation was made by Watson et al. (1974) on loblolly pine and hardwood leaf litter.

According to the Sorensen Index of similarity, the three kinds of leaves shared a high percentage of species. However, the frequency of occurrence of various fungi were different on the three types of leaves. On *Q. germana* and *Q. sartorii*, the dominant species was *B. rhombica*. By contrast, on *L. styraciflua* leaves, *B. rhombica* disappeared rapidly during the early stages of decay. The dominant species on *L. styraciflua* was *C. scoparium*. These results suggest a selective effect of the host leaves on the fungal flora. The chemical composition on leaf tissues may have important effects on fungi development (Swift, 1976).

Although *B. rhombica* was found on green leaves and fresh fallen leaves, this species appeared to prefer senescent and well-decayed leaves. Similar results were obtained by Padney (1990) on guava leaves. On wild plants Kiffer et al. (1981) and Rambelli et al. (1983) reported *B. rhombica* as a dominant fungus on leaf litter of tropical plants.

The presence of *C. scoparium* as the most abundant fungus on *L. styraciflua* leaf litter and the frequent detection of *Verticillium* spp. and *Fusarium* spp. may be related to the more rapid decay of this litter type. The genera *Verticillium* and *Fusarium* include some soft rot species, while *C. scoparium* has been reported as a phytopathogen causing leaf blight and damping-off on seedlings of ornamental plants (Westcott, 1960) and root decline of *Abies*, *Acacia* and *Liquidambar* hosts (Farr et al., 1989).

Ch. alabamensis, *E. gallsiae*, *C. assamica*, *C. kakombensis* and *S. procurvata* appeared regularly enough to be considered leaf litter-inhabiting fungi. All these fungi have a tropical and subtropical distribution (Ellis, 1971; Ellis, 1976; Rambelli et al., 1983).

The variation in the frequency of fungal species during the litter decomposition did not appear to be related to climatic conditions. The constant shedding of leaves along the year and the continuous canopy cover of the forest besides an almost constant mean temperature in this forest (Fig. 1), may retard evaporation from the deeper litter layers. Consequently, it is possible that the microclimate created in the leaf litter layer reduce the effect of weather changes on litter mycoflora. Other factors such as antagonism, competition, predation and chemical changes on the substrate must play an important role in determining the changes on the abundance of the fungal species.

Most of the species observed in this study were fungi imperfecti, especially dematiaceous forms. Hering (1965) and Rambelli et al. (1983) have pointed out the presence of dark mycelium species on leaf litter. Rambelli et al. (1983) concluded that a higher frequency of pigmented species exists on leaf litter than in soil. The advantages of fungal pigmented structures on leaves have been discussed by Diem

Primary saprophytes (green ■ fresh fallen leaves)	Secondary saprophytes (leaf litter)
	decomposition progress early middle advanced
	Leaf litter fungi
<i>Alternaria alternata</i>	<i>Beltrania rhombica</i> ¹
<i>Alternaria tenuissima</i>	<i>Codinaea assamica</i>
<i>Cladosporium cladosporioides</i>	<i>Cryptophiale kakombensis</i>
<i>Cladosporium herbarum</i>	<i>Cylindrocladium scoparium</i> ¹
<i>Curvularia lunata</i>	<i>Chalara alabamensis</i>
<i>Epicoccum purpurascens</i>	<i>Ellisiopsis gallestiae</i>
<i>Olpitrichum macrosporum</i>	<i>Pestalotiopsis maculans</i>
<i>Pestalotiopsis maculans</i>	<i>Subulispora procurvata</i>
<i>Rhinochadiella sp</i>	
<i>Tubakia dryina</i>	
	Soil fungi
	<i>Fusarium spp</i>
	<i>Penicillium spp</i>
	<i>Trichoderma viride</i>
	<i>Mucor hiemalis</i>
	<i>Verticillium sp</i>

Table 6 - General fungal succession pattern on *Quercus germana*, *Q. sartorii* and *Liquidambar styraciflua* leaves.

¹ Only on *Q. germana* and *Q. sartorii*. ² Only on *L. styraciflua*.

Tableau 6 - Modèle général de la succession fongique pendant la décomposition des feuilles de *Quercus germana*, *Q. sartorii* et *Liquidambar styraciflua*.

¹ Seulement sur *Q. germana* et *Q. sartorii*. ² Seulement sur *L. styraciflua*.

(1971). The thicker hyphal walls make them more resistant to bacterial lysis and depredation.

On the other hand the fungal potential for leaf component utilization, must influence the occurrence of certain species. White et al. (1948) reported many dark-pigmented fungi able to break down cellulose. Kjoller & Struwe (1980) isolated micro-fungi on decomposing red alder (*Alnus glutinosa*) leaves and tested their ability to grow on different substrates. They found that the dematiaceous *Phoma* spp. and *Cladosporium* spp. had high potentials for cellulose and starch utilization. These results suggest that the high frequency of dematiaceous species on leaf litter could be related to their ability to decompose leaf litter material. Unfortunately, the physiology of most fungi associated with the leaf litter is unknown.

A scheme of the fungal succession is presented in table 6. In general, the mycoflora succession pattern on the three leaves studied is like that mentioned by Hudson (1968) on deciduous leaves. As most of the leaf litter fungal successions described, Deuteromycetes species were observed since early decomposition stages, in contrast, Zygomycetes species were infrequent and appeared on late decay stages. This gives support to Webster's observations (1957) that Mucorales play little if any part in the decomposition of plant remains above the soil.

Regarding Basidiomycetes, mycelia with clamp connections were occasionally observed on damp chambers. The absence of Basidiomycetes in the isolation media may be due to their low capacity to grow on synthetic media.

Fungal species reported on leaf litter from temperate and tropical forests are the same as those observed on cloud forest leaf litter mycoflora on fresh fallen leaves and late decay. Nevertheless throughout the decomposition and on advanced decay stages there is a group of leaf litter-inhabiting fungi characteristic of tropical forests plant remains.

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